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Differential Potencies for Endogenous Dynorphins Indicate Functional Selectivity at the Delta Opioid Receptor

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Introduction

Opioid receptors (ORs) are G-protein Coupled Receptors (GPCRs), which mediate analgesia, tolerance, withdrawal, GI transit. Classically, ORs couple inhibitory $G\alpha_{i/o}$ proteins and recruit β-arrestin – a multifaceted scaffold molecule implicated in opioid mediated effects including tolerance, constipation, dysphoria and naseua [1,2]. Upon activation β-arrestin and $G\alpha_{i/o}$ induce downstream signaling responses such as reduced cAMP levels. Recent drug discovery efforts identified several functionally selective exogenous opiates which prefer certain signaling pathways at a given receptor – such as Gα stimulation – to others – such as β-arrestin recruitment and generate desired pharmacological properties [3,4]. Noting that most of the 20+ endogenous opioid peptides are non-selective and some opiates display functional selectivity, two important points emerge. First, endogenous and exogenous ligands, such as those used during studies, do not necessarily generate the same effects. Second, two different endogenous opioid peptides may differentially activate a given receptor. Dynorphin A (DynA) and Dynorphin B (DynB) are considered κOR agonists, despite binding to the δOR at 1.29nM and 3.39 nM [1], respectively. The Dynorphins start with the 5 amino acid Leu-enkephalin (Leu-Enk) sequence – YGGFL – traditionally considered a δOR agonist followed by distinct *C*-terminal sequences. Thus, we ask: Do Dynorphin A (1-17), Dynorphin B (1-13) and Leu-enkephalin induce functionally selective signaling at the δOR?

Results and Discussion

We assessed DynA, DynB and Leu-Enk signaling for two ubiquitous GPCR signals – Gα activation and β-arrestin recruitment – *in vitro*. GTPγS stimulation assays were performed using CHO cells expressing the δOR, analogous to previous reports [5]. β-Arrestin-2 recruitment assays (DiscoveRx) were performed by manufacturer's protocol. We found DynA, DynB and Leu-Enk display different potency rank orders for Gα activity and β-arrestin recruitment at the δOR (Table 1). DynB more potently activates Gα signaling than β-arrestin2 recruitment with EC50 values of 83 nM and 230 nM, respectively. In contrast, DynA more potently recruits β-arrestin than DynB with EC50 values of 83 and 328 nM, respectively (Table 1). A reversal in rank order between DynA and DynB likely indicates functional selectivity, such that each

Table 1. In Vitro Signaling Profile of Endogenous Opioids at δOR. DynA and DynB show distinct rank orders for arrestin-recruitment, GTPgS and cAMP; in addition DynA and DynB show distinct efficacies for cAMP inhibition relative to Leu-Enk. β-arrestin2 recruitment (n= 1), cAMP (n=3) and GTPyS (n=2) assays performed as referenced in text.

Ligand	*β-arrestin Recruitment*		*GTPγS*		*cAMP*	
	EC 50	*Emax*	*EC 50*	*Emax*	*EC 50*	*Emax*
Dynorphin A(1-17)	83	1.0	488	1.04	21	0.54
Dynorphin B(1-13)	330	1.0	383	1.04	122	0.52
Leu-Enkephalin	17	1.0	1.86	1.00	5.3	1.00

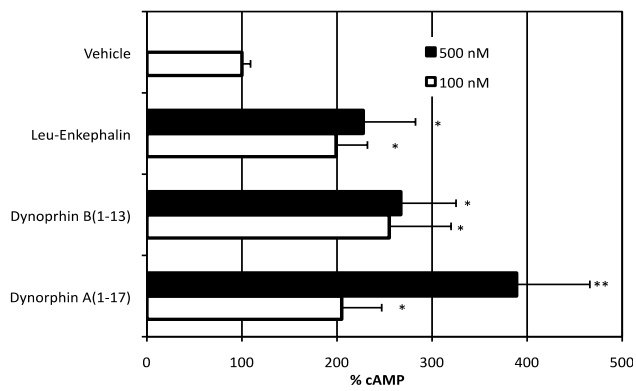

*Figure 1. δOR Mediated AC Super Activation. Cells were treated with vehicle or ligand for 24 hours - refreshing media every 8 hours - then AC was stimulated with 10 mM forskolin. Percentage of basal cAMP increase relative to vehicle was graphed and analyzed with a two-taliled t-test *p < .05, **p < .01.*

ligand likely induces distinct δOR conformations. Thus a modest 3-fold potency difference, may lead to more pronounced differences downstream.

Next we probed each ligand for forskolin-stimulated cAMP inhibition - a well-established Gα dependent pathway [6]. Acute treatment of DynA and Leu-Enk yield IC_{50} values of 21 nM and 5nM, respectively (Table 1). However, DynB exhibits a ~10 fold less potent response (IC_{50}=122 nM). DynB is a less potent agonist in the forskolin induced cAMP inhibition compared to DynA and Leu-Enk, whereas all display similar potency in the GTPγS assay. Based on differential cAMP inhibition potencies and efficacies, we predicted these peptides would differentially effect receptor mediated Gα regulation.

At the δOR, chronic opioid treatment causes a compensatory increase in basal cAMP levels, referred to as *Adenylyl Cyclase (AC) super activation*. AC super activation may play a physiological role in developing opioid tolerance, dependence and withdrawal [5,6]. Chronic treatment with DynA led to greater AC super activation than treatment with DynB or Leu-Enk, which similarly induce AC super activation (Figure 1). This upregulation in forskolin-stimulated cAMP indicates that DynA and DynB induce distinct receptor regulatory events. Differences in AC super activation by endogenous ligands could be important in the physiological roles of disease and require further *in vivo* study. Thus, *in vitro* assays show endogenous opioid peptides with differing (or absent) *C*-terminal tails induce distinct changes in the activity of these peptides. Therefore, we show three endogenous opioids induce distinct signaling and regulatory outcomes *in vitro*. Further studies are required to understand the differences in Gα activation, other cellular regulatory changes and potential uses as a peptide scaffold for drug design.

Acknowledgments

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